

A circular black and white stamp. The text "OIP" is at the top, "JCI" is at the top right, and "PATENT & TRADEMARK OFFICE" is at the bottom. In the center, the date "AUG 25 2005" is stamped.



2kb FOR THE EXPANDED MAP
2kb FOR THE TOP RESTRICTION MAP

REPLACEMENT SHEET

SUBCLONES OF DNaseB2 AND ITS ABILITY TO PRODUCE ACTIVE NUCLEASE

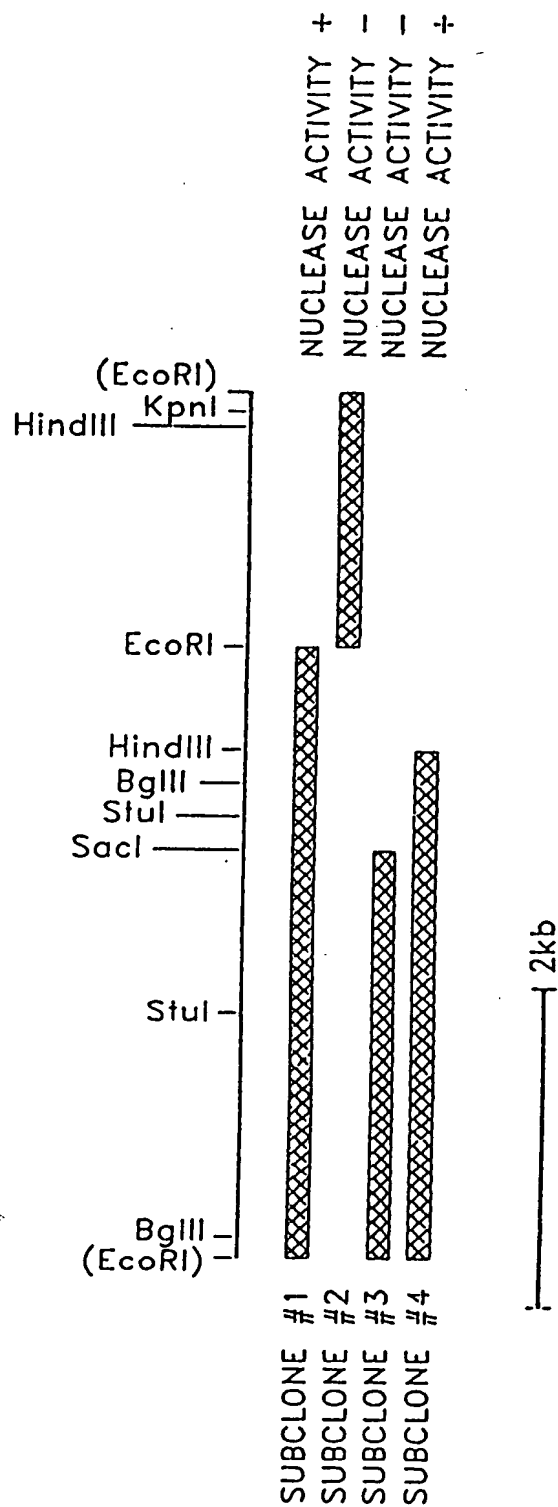


FIG. 2

REPLACEMENT SHEET

FIG. 3A

(SEQ ID NO: 8, 9)

1 GACAACGCCTTCTTTTCTCCTTACTATCTCTTAAATTTTCATATTTTAAAAAACTATTGATAAACTAGTTAAGTAAGCGTATCTATGGTTAGT
CTGTTGCCGAAGAAAAAGAGGAATGATACAGGAAATAAAAGTATAAAAAATTTTGTGATACTATTGATCAATTCATTCGCATATGATACCAATCA 100

a:
b:
c:

101 TAGCGAAATTAGAAAAGAGGACAAGCATATGAATCTACTTGGATCAAGACGGGTTTTTCTAAAAATGTCGGCTAGTAAAATTTTCAATGGTAGCTCTT
ATCGCTTAATCTTTCTCCTGTTCTGTTACTTATAGTGAACCTAGTTCTGCCCAAAAAAGATTTTACAGCCGATCATTTTAAAAGTTACCATCGAGAA 200

a:
b:
c:

MetAsnLeuLeuGlySerArgArgValPheSerLysLysCysArgLeuValLysPheSerMetValAlaLeu

201 GTATCAGCCACAATGGCTGTAACAACAGTCACACTTGAAATACTGCACTGGCAGCAGAACACAGGTCCTAAATGATGTTGTTCTAAATGATGGCGCAA
CATAGTCGGTGTACCGACATTGTTGTCAGTGTGAACCTTTATGACGTGACCGTGCTGTTGTCGTCAGAGTTTACTACAACAAGATTACTACCGCGTT 300

a:
b:
c:

ValSerAlaThrMetAlaValThrThrValThrLeuGluAsnThrAlaLeuAlaArgGlnThrGlnValSerAsnAspValValLeuAsnAspGlyAlaSer

301 GCAAGTACCTAAACGAAGCATTAGCTTGCACATTCAATGACAGTCCTAACTATTACAAAACCTTTAGGTACTAGTCAGATTACTCCAGCACTCTTTCCTAA
CGTTCATGGATTTCCTTCGTAATCGAACCTGTAAGTTACTGTGACGATTGATAATGTTTGAATCCATGATCAGTCTAATGAGGTCGTGAGAAAGGATT 400

a:
b:
c:

LysTyrLeuAsnGluAlaLeuAlaTrpThrPheAsnAspSerProAsnTyrTyrLysThrLeuGlyThrSerGlnIleThrProAlaLeuPheProLys

FIG. 3B

FIG. 3B

401
AGCAGGAGATATTCTCTATAGCAAATTAGATGAGTTAGGAAGGACGCGTACTGCTAGAGGTACATTGACTTATGCCAATGTTGAAGGTAGCTACGGTGTT
TCGTCCTCTATAAGAGATATCGTTTAATCTACTCAATCCTTCCTCGCATGACGATCTCCATGTAAGTGAATACGGTTACAACCTCCATCGATGCCACAA

501
AGACAATCTTTCCGGTAAAAATCAAAACCCCGCAGGATGGACTGGAAACCCCTAATCATGTCAAATATAAAATTGAATGGTTAAATGGTCTATCTTATGTCC
TCTGTTAGAAAGCCATTTTACTTTTGGGGCGCTACCTGACCTTTGGGATTAGTACAGTTTATATTTTAACCTACCAATTTACCAGATAGAATACAGC

601
GAGATTCTCGAATAGAAGTCATCTCATTCAGATAGTCTCGGTGGAGATGCACTCAGAGTCAATGCCGTTACAGGAACACGTACCCAAAATGTAGGAGG
CTCTAAAGACCTTATCTTCAGTAGAGTAACGTCTATCAGAGCCACCTCTACGTGAGTCTCAGTTACGGCAATGTCCTTGTCATGGGTTTACATCCTCC

701
TCGTGACCAAAAAGCGGCATGCGCTATACCGAACAAAGAGCTCAAGAATGGTTAGAAGCAAATCGTGATGGCTATCTTTATTATGAAGTCGCTCCAATC
AGCACTGGTTTTTCCGCCGTACCGGATATGGCTTGTTTCTCGAGTTCTTACCAATGTTTCGTTTAGCACTACCGATAGAAATAATACTTCACGAGGTTAG

801

a:
b:
c:

a:
b:
c:

a:
b:
c:

a:
b:
c:

REPLACEMENT SHEET

FIG. 3C

H C M C
 i Av b Av
 n li
 f uJ
 I II I II
 /
 801 TACAACGCAGACGAGTTGATTCCAAGAGCTGTCGGTATCAATGCAATCTTCTGATAATACCATCAACGAGAAAGTATTAGTTTACAACACAGCTAATG 900
 ATGTTGCGTCTGCTCAACTAAGGTTCTCGACAGCACCATTAGTTACGTTAGAAGACTATTATGGTAGTTGCTCTTTCATAATCAAATGTTGTGTCGATTAC

a:
 b:
 c: TyrAsnAlaAspGluLeuIleProArgAlaValValValSerMetGlnSerSerAspAsnThrIleAsnGluLysValLeuValTyrAsnThrAlaAsnGly -

C M C C s C
 v M vR vH MM vMaMSAv
 i s is ia an isIstli
 J e Qa Jc el JeIeuuJ
 I I II II IIIIIII
 /
 901 GCTACACCATTAACTACCATAACGGTACCTACTCAAAAATAATACCAAAAGGCTAGACCTCTGCTCACTAGGCCTAGCTTTTACATCAAAAAAGCA 1000
 CGATGTGGTAATTGATGGTATTGCCATGTGGATGAGTTTTTATTATGGTTTTCCGATCTCGAGACGAGTGATCCGGATCGAAAAATGTAGTTTTTTCGT

a:
 b:
 c: TyrThrIleAsnTyrHisAsnGlyThrProThrGlnLysEnd

B
 c
 M e
 s f
 e I
 I X
 T
 S
 p
 E
 I
 1001 ATGACTATAGAAAGTAAAAATACTAGAAAAAGCAATGATGCCGTCATTGCTTTTTATGAATTTGTGCAAAAAGCAAAAAAGC 1083
 TACTGATATCTTTTATTTTATGATCTTTTTCGTTACTAACGCCAGTAACGAAAAATACTTAAACACGTTTTTCGTTTTTTCG

a:
 b:
 c: MetAsnLeuCysLysLysGlnLysSer -
 MetIleAlaValIleAlaPheTyrGluPheValGlnLysAlaLysLys??? -

ENZYMES THAT DO CUT:

AccI	AflIII	AluI	AlwI	BanII	BceI	BglI	Bsp1286I	BsrI	CviJI	CviQI	DdeI	DpnI
DraI	Eco3II	EcoNI	Fnu4HI	FokI	SsuI	MaeI	MaeIII	HqaI	HgiAI	HhaI	HinfI	HinPI
MseI	MaeII	MseIII	MboII	MluI	MmeI	MnlI	MseI	MdeI	MlaIII	HspAI	PleI	RsaI
SacI	Sau3AI	SfaNI	SpeI	SphI	StuI	ThaI	Tsp45I	TspEI	Tth111III	Uba26I	XcaI	

ENZYMES THAT DO NOT CUT:

AatII	AflII	AhaII	AlwNI	AocI	ApaI	ApaII	Asp700I	Asp718I	AsuII	AvaI	AvaII	AvrII
BalI	BamHI	BanI	BbeI	BbvI	BbvII	BclI	BglIII	BsmI	BspHI	BspMI	BspMII	BssHI
BstEII	BstHI	BstXI	CfrI	Cfr10I	Clal	DraII	DraIII	DsaI	Eco47III	Eco57I	Eco78I	EcoRI
EcoRII	EcoRV	EspI	FinI	FinI	FspI	GdiII	HaeIII	HgiEII	HincII	HindIII	HpaIO	HpaII
MphI	KpnI	Ksp632I	MfeI	HaeI	MarI	NciI	NcoI	NheI	NlaII	NotI	NruI	NsiI
Msp6II	PflMI	PmaCI	PpuMI	PssI	PstI	PvuI	PvuII	RsrII	SacII	SalI	Sau96I	ScaI
SciI	ScrFI	SeaI	SfiI	SmaI	SmaII	SplI	SsoII	SspI	StyI	TaqI	TaqII	TaqII
Tth111I	VspI	XbaI	XhoI	XhoII	XmaI	XmaIII						

REPLACEMENT SHEET

AMINO ACID SEQUENCE OF CLONED S. PYOGENES DNase

R-Q-T-Q-V-S-N-D-V-V-L-N-D-G-A-S-K-Y-L-N-
E-A-L-A-W-T-F-N-D-S-P-N-Y-Y-K-T-L-G-T-S-
Q-I-T-P-A-L-F-P-K-A-G-D-I-L-Y-S-K-L-D-E-
L-G-R-T-R-T-A-R-G-T-L-T-Y-A-N-V-E-G-S-Y-
G-V-R-Q-S-F-G-K-N-Q-N-P-A-G-W-T-G-N-P-N-
H-V-K-Y-K-I-E-W-L-N-G-L-S-Y-V-G-D-F-W-N-
R-S-H-L-I-A-D-S-L-G-G-D-A-L-R-V-N-A-V-T-
G-T-R-T-Q-N-V-G-G-R-D-Q-K-G-G-M-R-Y-T-E-
Q-R-A-Q-E-W-L-E-A-N-R-D-G-Y-L-Y-Y-E-V-A-
P-I-Y-N-A-D-E-L-I-P-R-A-V-V-V-S-M-Q-S-S-
D-N-T-I-N-E-K-V-L-V-Y-N-T-A-N-G-Y-T-I-N-
Y-H-N-G-T-P-T-Q-K

(SEQ ID NO: 9)

FIG. 4

REPLACEMENT SHEET

PCR OLIGONUCLEOTIDE

5' TAACGGATCCGAATCTACTTGGATCAAGACGGGTTTTTCT 3' (SEQ. ID NO: 2)

1 ATGGATCCGAATCTACTTGGATCAAGACGGGTTTTTCTAAAAAATGTCGGCTAGTAAAA 60
 TACCTAGGCTTAGATGAACCTAGTTCTGCCCAAAAAAGATTTTTTACAGCCGATCATTTT
 MetAspProAsnLeuLeuGlySerArgArgValPheSerLysLysCysArgLeuValLys

61 TTTTCAATGGTAGCTCTTGTATCAGCCACAATGGCTGTAACAACAGTCACACTTGAAAAT 120
 AAAAGTTACCATCGAGAACATAGTCGGTGTTACCGACATTGTTGTCAGTGTGAACCTTTA
 PheSerMetValAlaLeuValSerAlaThrMetAlaValThrThrValThrLeuGluAsn

121 ACTGCACTGGCAGCACAACACAGGTCTCAAATGATGTTGTTCTAAATGATGGCGCAAGC 180
 TGACGTGACCGTGCTGTTTGTGTCCAGAGTTTACTACAACAAGATTTACTACCGCGTTTCG
 ThrAlaLeuAlaArgGlnThrGlnValSerAsnAspValValLeuAsnAspGlyAlaSer

181 AAGTACCTAAACGAAGCATTAGCTTGGACATTCAATGACAGTCCTAACTATTACAAAAC 240
 TTCATGGATTGCTTCGTAATCGAACCTGTAAGTTACTGTCAGGATTGATAATGTTTTGA
 LysTyrLeuAsnGluAlaLeuAlaTrpThrPheAsnAspSerProAsnTyrTyrLysThr

241 TTAGGTACTAGTCAGATTACTCCAGCACTCTTTCCTAAAGCAGGAGATATTCTCTATAGC 300
 AATCCATGATCAGTCTAATGAGGTCGTGAGAAAGGATTTTCGTCCTCTATAAGAGATATCG
 LeuGlyThrSerGlnIleThrProAlaLeuPheProLysAlaGlyAspIleLeuTyrSer

301 AAATTAGATGAGTTAGGAAGGACGCGTACTGCTAGAGGTACATTGACTTATGCCAATGTT 360
 TTTAATCTACTCAATCCTTCCTGCGCATGACGATCTCCATGTAAGTGAATACGGTTACAA
 LysLeuAspGluLeuGlyArgThrArgThrAlaArgGlyThrLeuThrTyrAlaAsnVal

361 GAAGGTAGCTACGGTGTTAGACAATCTTTCGGTAAAAATCAAAACCCCGCAGGATGGACT 420
 CTTCATCGATGCCACAATCTGTTAGAAAGCCATTTTGTAGTTTGGGGCGTCCTACCTGA
 GluGlySerTyrGlyValArgGlnSerPheGlyLysAsnGlnAsnProAlaGlyTrpThr

421 GGAAACCCTAATCATGTCAAATATAAAATTGAATGGTTAAATGGTCTATCTTATGTCGGA 480
 CCTTTGGGATTAGTACAGTTTATATTTTAACTTACCAATTTACCAGATAGAATACAGCCT
 GlyAsnProAsnHisValLysTyrLysIleGluTrpLeuAsnGlyLeuSerTyrValGly

FIG. 5A

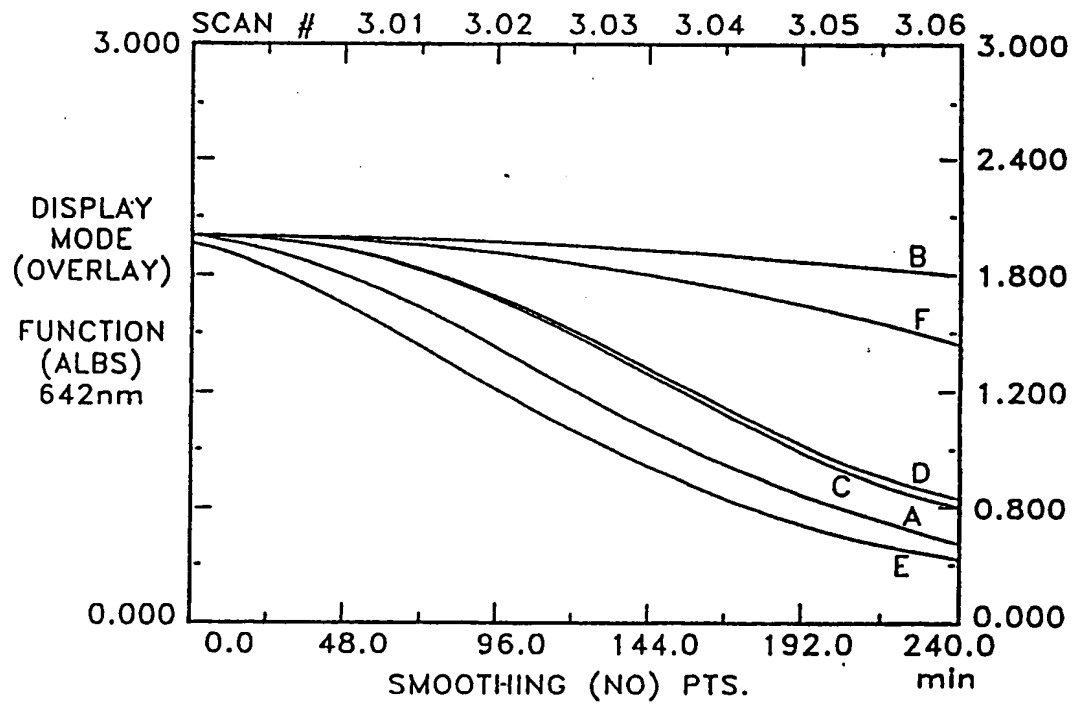
REPLACEMENT SHEET

481 GATTTCTGGAATAGAAGTCATCTCATTGCAGATAGTCTCGGTGGAGATGCACTCAGAGTC 540
 CTAAAGACCTTATCTTCAGTAGAGTAACGTCTATCAGAGCCACCTCTACGTGAGTCTCAG
 AspPheTrpAsnArgSerHisLeuIleAlaAspSerLeuGlyGlyAspAlaLeuArgVal
 541 AATGCCGTTACAGGAACACGTACCCAAAATGTAGGAGGTCGTGACCAAAAAGGCGGCATG 600
 TTACGGCAATGTCCTTGTGCATGGGTTTACATCCTCCAGCACTGGTTTTTCCGCCGTAC
 AsnAlaValThrGlyThrArgThrGlnAsnValGlyGlyArgAspGlnLysGlyGlyMet
 601 CGCTATACCGAACAAGAGCTCAAGAATGGTTAGAAGCAAATCGTGATGGCTATCTTTAT 660
 GCGATATGGCTTGTCTCGAGTTCTTACCAATCTTCGTTTAGCACTACCGATAGAAATA
 ArgTyrThrGluGlnArgAlaGlnGluTrpLeuGluAlaAsnArgAspGlyTyrLeuTyr
 661 TATGAAGTCGCTCCAATCTACAACGCAGACGAGTTGATTCCAAGAGCTGTCGTGGTATCA 720
 ATACTTCAGCGAGGTTAGATGTTGCGTCTGGTCAACTAAGTTCTCGACAGCACCATAGT
 TyrGluValAlaProIleTyrAsnAlaAspGluLeuIleProArgAlaValValValSer
 721 ATGCAATCTTCTGATAATACCATCAACGAGAAAGTATTAGTTTAGAACAGAGCTAATGGC 780
 TACGTTAGAAGACTATTATGGTAGTTGCTCTTTCATAATCAAATGTTGTGTCGATTACCG
 MetGlnSerSerAspAsnThrIleAsnGluLysValLeuValTyrAsnThrAlaAsnGly
 781 TACACCATTAACCTACCATAACGGTACACCTACTCAAAAATAATACCAAAAGGCTAGACCT 840
 ATGTGGTAATTGATGGTATTGCCATGTGGATGAGTTTTTATTATGGTTTTCCGATCTGGA
 TyrThrIleAsnTyrHisAsnGlyThrProThrGlnLysEndTyrGlnLysAlaArgPro
 841 CTGCTCACTAGGCCTAGCTTTTTACATCAAAAAAGCAATGACTATAGAAAGTAAAAATA 900
 GACGAGTGATCCGGATCGAAAAATGTAGTTTTTTTCGTTACTGATATCTTTCATTTTTAT
 LeuLeuThrArgProSerPheLeuHisGlnLysLysGlnEnd
 901 CTAGAAAAAGCAATGATTGCCGTCATTGCCCGGGTCGACCCGG 944 (SEQ. ID NO: 1)
 GATCTTTTTTCGTTACTAACGGCAGTAACGGGGCCAGCTGGGCC
 3' TCTTTTTTCGTTACTAACGGCAGTAACGGGGCCAGCTGGGCC 5' (SEQ. ID NO: 3)

PCR OLIGONUCLEOTIDE

FIG. 5B

REPLACEMENT SHEET



A=STREPTONASE B
 B=STREPTONASE B + ANTIBODY
 C=DNase1-1
 D=DNase1-1 + ANTIBODY
 E=DNase B2-6
 F=DNase B2-6 + ANTIBODY

FIG. 6

REPLACEMENT SHEET

1 GACAACGCCCTTCTTTTTTCTCCTTCTCTCTTAAATTTTCATATTTTTTAAAAAACTATTGATAAACTAGTTAAGTAAGCGTATACTATGGTTAGT -35 -10
101 TAGCGAAATTAGAAAAGAGGACAAAGCATATGAATCTACTTGGATCAAGACGGGTTTTTTTCTAAAAAATGTCGGCTAGTAAAAATTTTCAATGCTAGCCTT

(SEQ. ID NO: 10)

CONSENSUS SEQUENCE OF ESCHERICHIA COLI PROMOTER REGION:

-35 -10
tcTTGACat TATAat

FIG. 7

REPLACEMENT SHEET

CORRELATION CURVE OF ACTIVITY ASSAYS
BASED ON RECOMBINANT AND NATURAL
(STREPTONASE B) DNAase B

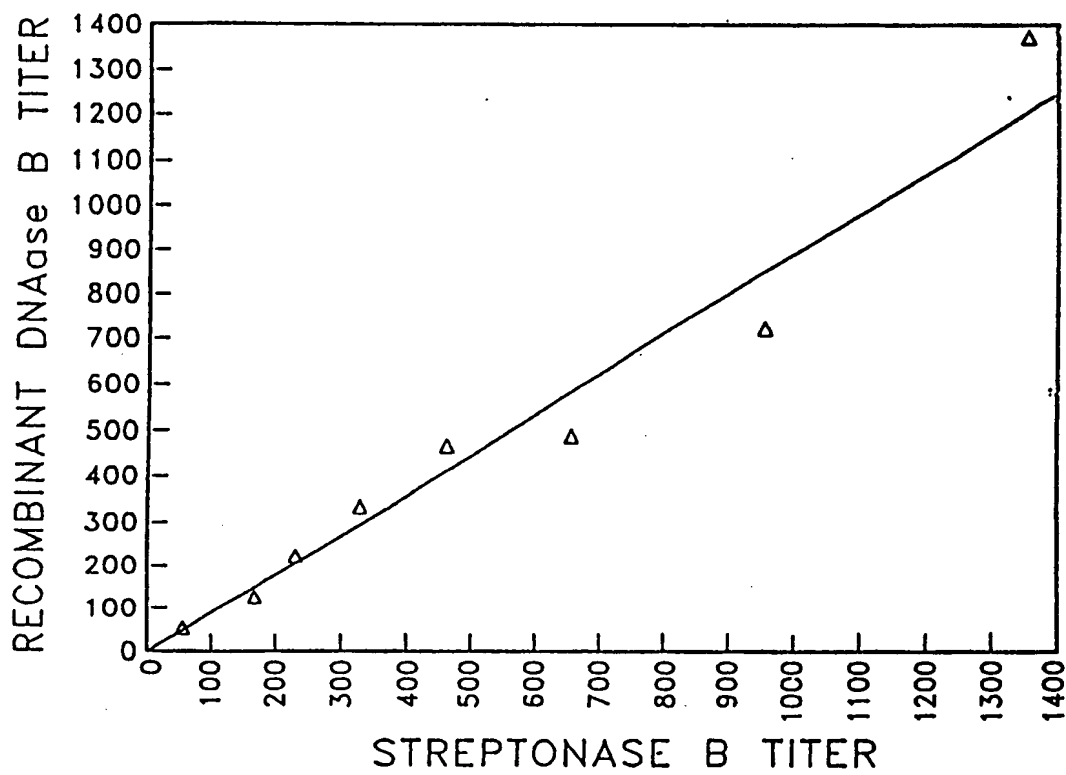


FIG. 8

MITOGENIC ASSAY WITH MOUSE LYMPHOCYTES

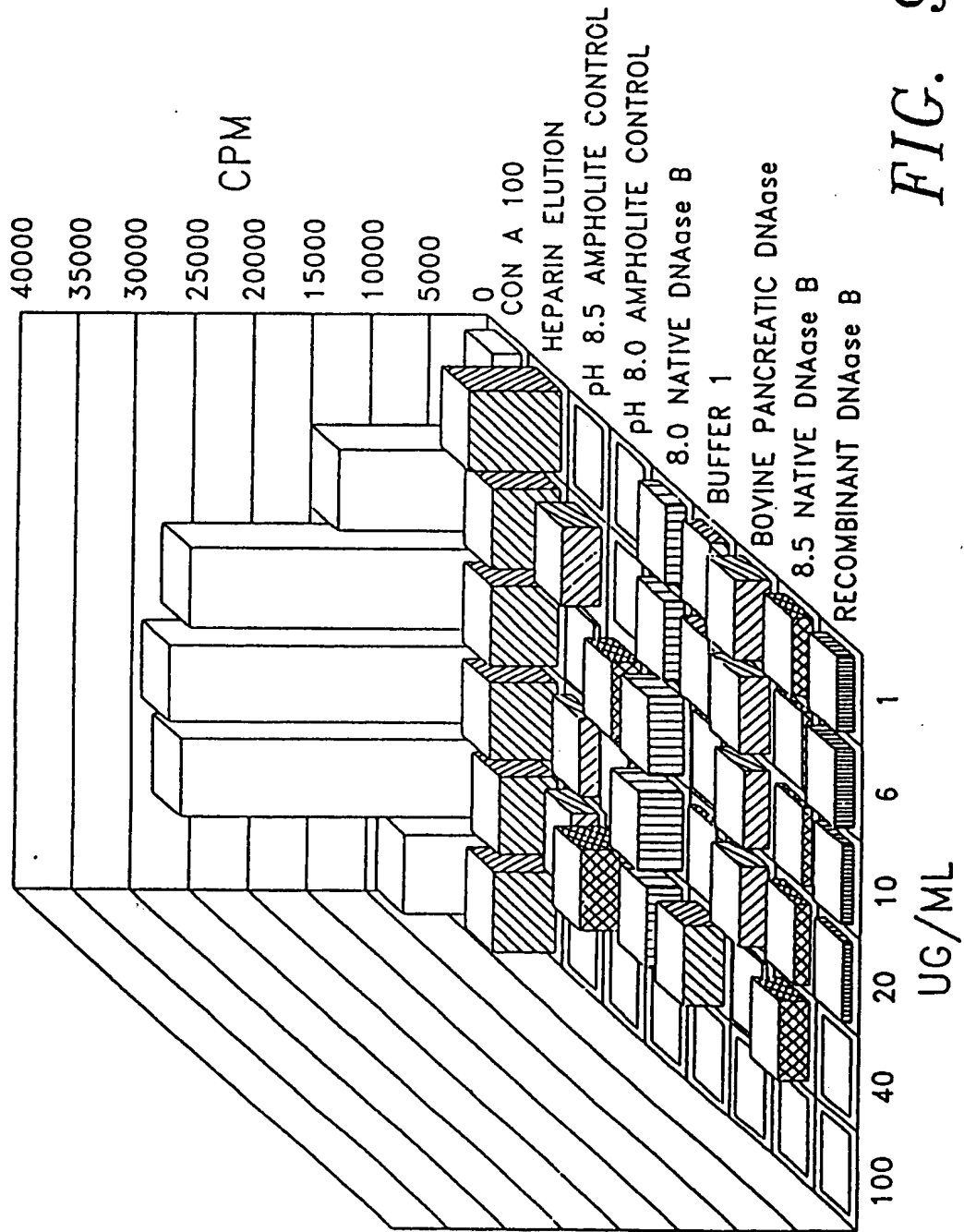


FIG. 9

REPLACEMENT SHEET

SEQUENCE OF CONSTRUCTION PRODUCING DNASE B PROCESSED IDENTICALLY TO NATURAL DNASE B

PCR OLIGONUCLEOTIDE (SEQ ID NO: 12)

5' AGGCAATGGATCCGAACCTGCTGGGTTCCCGTCGTGTTTTCTCCAAAAATGCCGTCTGGTTAAATTCTCCAT
ATGGATCCGAACCTGCTGGGTTCCCGTCGTGTTTTCTCCAAAAATGCCGTCTGGTTAAATTCTCCAT 60
TACCTAGGCTTAGATGAACCTAGTTCTGCCAAAAAGATTTTTACAGCCGATCATTTTAAAGTTA
MetAspProAsnLeuLeuGlySerArgArgValPheSerLysLysCysArgLeuValLysPheSerMet -
GGTTGCTCTGGTTCCCGTACCATGGCTGTTACCACCGTTACCCTGGAAAAACACCGCTCT
GGTTGCTCTGGTTCCCGTACCATGGCTGTTACCACCGTTACCCTGGAAAAACACCGCTCT 120
61 CCATCGAGAACATAGTCGGTGTACCACGATTGTTGTCAGTGTGAACCTTTATGACGTGA
ValAlaLeuValSerAlaThrMetAlaValThrThrValThrLeuGluAsnThrAlaLeu -
GGCT***CAGACACAGGTCTCAAATGATGTTGTTGTAATGATGGCGCAAGCTTCATGGA
GGCT***CAGACACAGGTCTCAAATGATGTTGTTCTAAATGATGGCGCAAGCAAGTACCT 180
121 CCGTGCTGTTTCTCTCCAGAGTTTACTACAACAAGATTTACTACCGCGTTCGTTTCATGGA
AlaArgGlnThrGlnValSerAsnAspValValLeuAsnAspGlyAlaSerLysTyrLeu -
AAACGAAGCATTAGCTTGGACATTCAATGACAGTCCTAACTATTACAAAACCTTTAGGTAC 240
181 TTTGCTTCGTAATCGAACCTGTAAGTTACTGTCAGGATTGATAATGTTTTGAAATCCATG
AsnGluAlaLeuAlaTrpThrPheAsnAspSerProAsnTyrTyrLysThrLeuGlyThr -
TAGTCAGATTACTCCAGCACTCTTCTCTAAAGCAGGAGATATTCTCTATAGCAAATTAGA 300
241 ATCAGTCTAATGAGGTCGTGAGAAAGATTTCGTCTCTATAAGAGATATCGTTTAATCT
SerGlnIleThrProAlaLeuPheProLysAlaGlyAspIleLeuTyrSerLysLeuAsp -
TGAGTTAGGAAGGACCGTACTGCTAGAGGTACATTGACTTATGCCAATGTTGAAGGTAG 360
301 ACTCAATCCTTCTGCGCATGACGATCTCCATGTAAGTGAATACGGTTACAACCTCCATC
GluLeuGlyArgThrArgThrAlaArgGlyThrLeuThrTyrAlaAsnValGluGlySer -
CTACGGTGTAGACAATCTTTTCGGTAAAAATCAAAACCCCGCAGGATGGACTGGAACCC 420
361 GATGCCACAATCTGTTAGAAAGCCATTTTAGTTTTGGGGCGTCTACCTGACCTTTGGG
TyrGlyValArgGlnSerPheGlyLysAsnGlnAsnProAlaGlyTrpThrGlyAsnPro -
TAATCATGTCAAATATAAAATTGAATGGTTAAATGGTCTATCTTATGTCGGAGATTTCTG 480
421 ATTAGTACAGTTTATATTTAACTTACCAATTTACCAGATAGAATACAGCCTCTAAAGAC
AsnHisValLysTyrLysIleGluTrpLeuAsnGlyLeuSerTyrValGlyAspPheTrp -
GAATAGAAGTCATCTCATTTGCAGATAGTCTCGGTGGAGATGCACTCAGAGTCAATGCCGT 540
481 CTTATCTTCAGTAGAGTAACGTCTATCAGAGCCACCTCTACGTGAGTCTCAGTTACGGCA
AsnArgSerHisLeuIleAlaAspSerLeuGlyGlyAspAlaLeuArgValAsnAlaVal -

FIG. 10A

REPLACEMENT SHEET

541 TACAGGAACACGTACCCAAAATGTAGGAGGTCGTGACCAAAAAGGCGGCATGCGCTATAC 600
 ATGTCCTTGTGCATGGGTTTACATCCTCCAGCACTGGTTTTCCGCGGTACGCGATATG
 ThrGlyThrArgThrGlnAsnValGlyGlyArgAspGlnLysGlyGlyMetArgTyrThr -
 601 CGAACAAAGAGCTCAAGAATGGTTAGAAGCAAATCGTGATGGCTATCTTTATTATGAAGT 660
 GCTTGTCTCGAGTTCTTACCAATCTTCGTTTAGCACTACCGATAGAAATAATACTTCA
 GluGlnArgAlaGlnGluTrpLeuGluAlaAsnArgAspGlyTyrLeuTyrTyrGluVal -
 661 CGCTCCAATCTACAACGCAGACGAGTTGATTCCAAGAGCTGTCGTGGTATCAATGCAATC 720
 GCGAGGTTAGATGTTGCGTCTGCTCAACTAAGGTTCTCGACAGCACCATAGTTACGTTAG
 AlaProIleTyrAsnAlaAspGluLeuIleProArgAlaValValValSerMetGlnSer -
 721 TTCTGATAATACCATCAACGAGAAAGTATTAGTTTACAACACAGCTAATGGCTACACCAT 780
 AAGACTATTATGGTAGTTGCTCTTTCATAATCAAATGTTGTGTCGATTACCGATGTGGTA
 SerAspAsnThrIleAsnGluLysValLeuValTyrAsnThrAlaAsnGlyTyrThrIle -
 781 TAACTACCATAACGGTACACCTACTCAAAAATAATACCAAAAGGCTAGACCTCTGCTCAC 840
 ATTGATGGTATIGCCATGTGGATGAGTTTTTATTATGGTTTTCCGATCTGGAGACGAGTG
 AsnTyrHisAsnGlyThrProThrGlnLysEnd (SEQ ID NO: 15)
 841 TAGGCCTAGCTTTTTACATCAAAAAAGCAATGACTATAGAAAGTAAAAATACTAGAAAA 900
 ATCCGGATCGAAAAATGTAGTTTTTTTCGTTACTGATATCTTTCATTTTTATGATCTTTT
 3' TCTTTT
 901 AGCAATGATTGCCGTCATTGCCCCGGGTCGAC (SEQ ID NO: 14) 936
 TCGTTACTAACGGCAGTAACGGGGCCCAGCTG
 TCGTTACTAACGGCAGTAACGGGGCCCAGCTGGGCC 5' (SEQ ID NO: 13)
 PCR OLIGONUCLEOTIDE

FIG. 10B